

REMARKS

I. Status of the Claims

Claims 1, 2, 5-11, and 13 are pending. The limitations of claim 4 have been incorporated into claims 1 and 13. Accordingly, no new matter is added with these amendments.

II. Response to Specific Comments in the Advisory Action

The Scope of Claim 4 Has Not Changed; Claim 1 Has the Scope of Previous Claim 4

On page 2 of the Advisory Action, the Examiner asserts that the claims have been amended so the scope of the invention is changed. "This would raise potential new grounds of rejection; so further search and/or consideration is required. Thus, the amendment does not simplify the issues of the rejections and further does not place the application in better condition for allowance."

Applicants respectfully point out that the scope of the invention has not changed with respect to rejected claim 4 because claim 1 has been amended to incorporate the limitations of claim 4. This, accordingly, would not raise a potential new ground for rejection and further search and/or consideration would not be required; the issues would be simplified; this would place the application in better condition for appeal or allowance.

The Examiner will note that, originally, claim 4 recited the following:

The method of claim 1 wherein said cells are cultured according to steps (a) through (d) for at least seven days at each step.

The present claims simply incorporate this text into claim 1, which now recites "wherein said cells are cultured according to steps (a) through (d) for at least seven days at each step." Accordingly, it is plain that the scope of claim 4 is not changed.

The amendment, therefore, should have been entered.

III. The Rejections

A. Rejection Under 35 U.S.C. § 112, Second Paragraph

On pages 3 and 4 of the final Office Action, claims 3, 7, and 8 are rejected as indefinite. Applicants traverse the rejection.

The Examiner insists that the term “N2” is inappropriate. Reference to the term is “N2” is deleted.

In addition, on page 4 of the Office Action, the Examiner asserts that the metes and bounds of the following are unclear: “cells that are not *embryonic stem cells*, *embryonic germ cells*, or *germ cells*, and can differentiate into at least one cell type of each of the *endodermal*, *ectodermal*, and *mesodermal embryonic lineages*.” Applicants respectfully disagree.

The metes and bounds of the above phrases would be clear as all terms are well-accepted scientific terms. They are even found in Wikipedia. (See Appendix A, attached.) An embryonic stem cell would be known as a cell derived from the inner cell mass of a blastocyst and having the ability to differentiate into all the cells of the body, including germ cells, but not the extra-embryonic tissues of the developing embryo. Embryonic germ cells are known and accepted in the art as the primordial germ cells in the embryo that give rise to the germ cells of the post-natal organism. The term “germ cells” is understood to encompass egg and sperm. In addition, the three embryonic germ layers (endoderm, ectoderm, and mesoderm) are well-understood by the person of ordinary skill in the art. For the Examiner’s reference, Applicants also include Appendix B that demonstrates the common knowledge of these terms to the point where they are even found in succinct forms in a glossary. Appendix B also contains basic textbook information regarding these terms and related developmental biology.

Furthermore, precedent in the USPTO is that these terms are not indefinite. Applicants point out that, in allowed application 11/238,234 and in application 10/467,963, there has been no objection to these terms.

The Examiner is directed to these applications. A copy of the acceptable claims is attached as Appendix C.

Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

B. Rejections Under 35 U.S.C. § 103

1. Rejection Over WO 02/086073 (Studer) in View of U.S. 2003/0211605 (Lee)

On page 5 of the final Office Action, claims 1-7, 9, 11, and 13 are rejected on the grounds that they are unpatentable for the reasons of record. Applicants respectfully traverse the rejection.

The Studer/Lee Differentiation Protocol: All Factors Applied At Once to Single Phenotypic Cell Type

First, Applicants point out that both of the references teach the same differentiation procedures. The difference is that WO 02/086073 starts with nuclear transfer stem cells and U.S. 2003/0211605 starts with blastocyst-derived embryonic stem cells.

The differentiation protocol in these references involve five stages. Page 4 of WO 02/086073 shows the basic steps. In the first three stages, the cells are not exposed to any of the claimed factors. First, ES cells are allowed to form an embryoid body. Second, the embryoid body is placed in a new container with an attachment factor and grown for nine to sixteen days. Then, the embryoid body contains cells that express the neural marker nestin. (The reference refers to this as “nectin.”) The expression of nestin signals “commitment” to differentiate into cells of neural fate. These cells, therefore, are no longer ES cells. Then, in a fourth stage, the factors are applied. In this fourth stage, the committed nestin-positive cells are exposed to a mitogen (e.g., bFGF and BDNF), sonic hedgehog (SHH), and fibroblast growth factor 8 (FGF8) all at once. This results in specific types of neural cells in a process known in the art as “specification.” Accordingly, all of these factors act at the same time on a specific committed cell type.

Examiner's Rationale

In the paragraph spanning pages 6 and 7 of the Office Action, the Examiner hypothesizes that sequential addition of the factors would be an obvious modification of the prior art protocol.

...at the end of the final step, the culture medium contains the identical growth factors as those in Studer's to induce neuronal differentiation. By adding bGFG, FGF8, SHH, and BDNF in a different order from that of Studer's method would not result in any superior result because at the end the culture medium still contains the same growth factors and the same cultured ES cells, which would be induced to differentiate into neurons.

Emphasis added.

The Law Does Not Require a Superior Result

First, Applicants point out that patentability is not based on superior results. Applicants believe that the Examiner means to assert that the presently-claimed method would have been expected to produce the same result as the prior art method. Applicants do not agree for reasons addressed in detail below and explained in the attached Declaration of Dr. Catherine Verfaillie.

Claim 1 is Now Directed to a Seven-Day Incubation at Each Step

Adding the factors sequentially might produce the same result as simultaneous exposure if one were to add all factors within minutes, or perhaps even hours, of each other (although this has not been tested). But where each individual step is carried out for at least seven days, one would not reasonably have expected the same result as simultaneous exposure.

Applicants have incorporated the limitations of previous claim 4 into claim 1. The Examiner included claim 4 in the rejection but did not discuss this claim. The Examiner did not explain what would have motivated the person of ordinary skill in the art to drastically alter the Studer and Lee procedure by culturing for seven days at each step. Applicants submit that the person of ordinary skill would not have been motivated to practice this embodiment.

First, it is not reasonable to assume that all the growth factors would be present after 21 days in culture. All of the factors (a) – (c) are proteins. They would be expected to degrade in cell culture medium at 37° over such a prolonged period. But, even if they did persist in an active state, it is scientifically unreasonable, and contrary to established scientific principles, to believe that a cell produced by the claimed procedure would be the same as the cell produced with the prior art procedure. This is because the factors in the claimed method would act on four phenotypically discrete cell types while the factors in the prior art method act on one phenotypically discrete cell type. And this phenotypically discrete cell type is not even an ES cell. Applicants explain below.

At the End of the Claimed Procedure, the Culture Medium Likely Would Not Contain “the Same Cultured ES Cells”

The Examiner bases the rejection on the assumption that, after sequential exposure, all of the factors would act simultaneously on the “same cultured ES cell.” This is not a reasonable assumption and contrary to what would have been expected scientifically. As explained directly below, each factor would have been expected to induce a specific phenotypic effect. Accordingly, one would have expected to lose the original ES cell phenotype as soon as one exposed the cells to the first growth factor, i.e., fibroblast growth factor. The cell with the new phenotype would then be expected to express a further phenotype by exposure to the second two factors, and so on. Accordingly, at the end, the culture medium, even if it contains the same growth factors (which it most likely does not), would not have been expected to contain “the same cultured ES cells.

The Final Phenotype Would Have Been Expected to Depend on Whether Factors Are Added Sequentially or Simultaneously

The Examiner cites *KSR International v. Teleflex Inc.* (82 USPQ 2d 1385 (US2007)). This test was not disturbed by the Supreme Court in its rationale for the decision in *KSR*. In fact, the Supreme Court emphasized that an invention may be “obvious to try,” but to find a *prima facie* case of obviousness, “reasonable predictability” is required.

Performing the method sequentially, with sufficient time between each step, would not have reasonably predicted cells with the same phenotype. More likely than not, the cells should be different with the two methods.

In the cited art all factors are exposed to the same initial cell type (a neural-committed cell). But in the claimed method each factor acts on four phenotypically discrete cell types, i.e., the FGF8 and SHH act on the specific and discrete cell produced in step (a) by bFGF, the BDNF acts at the specific and discrete cell produced in step (b) by FGF8 and SHH, and the astrocytes act on the specific and discrete cell produced in step (c) by BDNF. Because one would expect four different phenotypic cell types, it is not reasonably predictable that the final cell type will be the same cell as produced by the prior art methods. Accordingly, the person of ordinary skill would not have been motivated to qualitatively and fundamentally modify the procedure as Applicants have done because they would not have known what to expect; the results were not reasonably predictable.

The Examiner is respectfully directed to the attached Declaration of Dr. Catherine Verfaillie, an expert in the field of stem cell research, and one of the inventors in the above-captioned application. Dr. Verfaillie explains the Applicants' position discussed above with more particularity and detail. She explains that the rejection is based on an incorrect scientific reasoning about how the cells would respond to each of the claimed factors when they are administered simultaneously as opposed to when they are administered sequentially. She also explains how sequential administration creates four discrete cell types that would

be phenotypically unique and would be expected to have a unique response to the growth factor to which it is exposed. She explains that the end product of sequential exposure cannot be reasonably predicted based on the results of simultaneous exposure to the same factors.

This explanation is found starting on page 2 of the Declaration. She also refers to an example of this principle in a reference (Snykers et al.) attached to the Declaration. She concludes that, in her opinion as an expert in the field, qualified to speak to what the person of ordinary skill in the art would have expected, this hypothetical person would not have been motivated to drastically alter the procedure of Studer and Lee as in the method now claimed because they would not have reasonably expected to produce the same results.

Studer Does Not Expose the Factors to ES Cells

Finally, Applicants point out that the prior art cells that are exposed to the factors are not even ES cells. They are neural progenitors. They express nestin. ES cells do not. Therefore, to the extent that the rejection is based on this assumption, the rejection should be reconsidered.

Summary Argument

Based on the Declaration and scientific reasoning by Dr. Verfaillie, Applicants submit that the person of ordinary skill would not have been motivated to change the procedure so drastically because the same end product would not be reasonably predictable. So, even if one had been motivated "to try" this approach, there was not a reasonable expectation of successfully producing the prior art cell.

The Examiner believes that with sequential addition the cell eventually is exposed to all factors and so one would expect the same end result. But, as Dr. Verfaillie has explained, each factor induces some phenotypic change on the cell to which it is exposed. At the end, one has a cell that has discrete phenotypic predecessors: a first predecessor not expressing nestin; a second predecessor produced by inducing a new phenotype with bFGF after bFGF; a third predecessor produced by inducing a new

phenotype with SHH and FGF8, and; a fourth predecessor produced by inducing a new phenotype with BDNF. By the time all factors are present, the cell, more likely than not, is different from the cell that Studer and Lee produced.

Moreover, the cells exposed to the factors in the prior art methods are not ES cells. They are neural committed cells.

For any or all of these reasons, it is not reasonable to expect the same end product if one uses the claimed approach instead of a simultaneous ("cocktail") approach.

For these reasons, Applicants believe that they have addressed the reasons for rejection and have adequately overcome the rejection. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

2. Rejection Over WO 02/086073 in View of U.S. 2003/0211605 and
Further in View of Song et al.

On page 9 of the Office Action, claims 1-11 and 13 are rejected on the grounds that they are unpatentable over WO 02/086073 and U.S. 2003/0211605 and further in view of Song et al. (*Methods in Molecular Biology*, 198:79-88 (2002)). The Examiner maintains the rejection for reasons of record. Applicants respectfully traverse the rejection.

Song is used to address claims directed to multipotent adult progenitor cells in bone marrow per claims 7-10. The basis for the rejection is that these are stem cells and, therefore, it is obvious to differentiate them into neurons. Applicants respectfully disagree.

Song does not compensate for the deficiencies of WO 02/086073 and U.S. 2003/0211605. These references are deficient against the currently amended claims for reasons give in detail above. Song does not compensate for these deficiencies. Song is not directed, as the Examiner recognizes, to any

differentiation protocol at all, but merely as teaching stem cells. Accordingly, the maintenance of the rejection over this combination of references is improper and should be withdrawn.

Applicants have adequately addressed the rejection of the claim over these references and overcome the rejection. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

C. Rejection of the Claims Under 35 U.S.C. § 112, First Paragraph

On page 10 of the Office Action, claims 7 and 8 have been rejected on the grounds that they do not comply with the written description requirement, indicating “this is a new matter rejection.” (Emphasis is in the original.) Specifically, the Examiner objects to the phrase “cells that are not embryonic stem cells, embryonic germ cells, or germ cells and can differentiate into at least one cell type of each of the endodermal, ectodermal, and mesodermal embryonic lineages.” The Examiner indicates that this was not disclosed in the specification and, thus, introduces new matter. Applicants respectfully traverse the rejection.

First, Applicants point out that there is precedent in the USPTO that contradicts the Examiner’s determination that this is a new matter rejection. Applicants direct the Examiner to Applicants’ related applications 11/238,234 and 10/467,963. The Examiner will note that in neither prosecution has the Examiner rejected the claims based on inadequate written description. Applicants incorporated the parent application (now U.S. Patent 7,015,037) into 11/238,234 and 10/467,963 as well as into the present application. See “Related Applications/Patents and Incorporation by Reference.” In an amendment in 11/238,234, Applicants pointed out support, for example, in the Summary of the Invention, paragraph spanning pages 8 and 9 and the first full paragraph of page 9. These pages are attached, for the Examiner’s convenience, as Appendix D. Accordingly, Applicants believe, and there is USPTO precedent, that the phrase to which the Examiner objects as new matter is not, in fact, new matter and is adequately described.

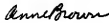
Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

IV. Conclusion

In view of the Applicants' amendments and discussion, Applicants believe that the pending claims are in condition for allowance. Early notification to that effect is respectfully requested. If it is believed that a further interview will expedite prosecution, the Examiner is invited to contact Applicants' attorney Anne Brown at 216-566-8921.

Applicants do not believe that any fees are due with this filing. In the event that fees are incurred, however the Commissioner is hereby authorized to charge any additional fees required to Deposit Account 20-0809. The applicant(s) hereby authorizes the Commissioner under 37 C.F.R. §1.136(a)(3) to treat any paper that is filed in this application which requires an extension of time as incorporating a request for such an extension.

Respectfully submitted,



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Appendix A

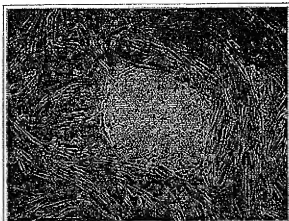
Embryonic stem cell

From Wikipedia, the free encyclopedia

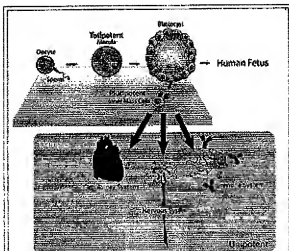
Embryonic stem cells (ES cells) are stem cells derived from the inner cell mass of an early stage embryo known as a blastocyst. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50–150 cells.

Embryonic Stem (ES) cells are pluripotent. This means they are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include each of the more than 220 cell types in the adult body. Pluripotency distinguishes ES cells from multipotent progenitor cells found in the adult; these only form a limited number of cell types. When given no stimuli for differentiation, (i.e. when grown *in vitro*), ES cells maintain pluripotency through multiple cell divisions. The presence of pluripotent adult stem cells remains a subject of scientific debate; however, research has demonstrated that pluripotent stem cells can be directly generated from adult fibroblast cultures.^[1]

Because of their plasticity and potentially unlimited capacity for self-renewal, ES cell therapies have been proposed for regenerative medicine and tissue replacement after injury or disease. However, to date, no approved medical treatments have been derived from embryonic stem cell research. Adult stem cells and cord blood stem cells have thus far been the only stem cells used to successfully treat any diseases. Diseases treated by these non-embryonic stem cells include a number of blood and immune-system related genetic diseases, cancers, and disorders; juvenile diabetes; Parkinson's; blindness and spinal cord injuries. Besides the ethical concerns of stem cell therapy (see *stem cell controversy*), there is a technical problem of graft-versus-host disease associated with allogeneic stem cell transplantation. However, these problems associated with histocompatibility may be solved using autologous donor adult stem cells or via therapeutic cloning.



Human embryonic stem cells in cell culture



Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.

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Research history and developments

Isolation and *in vitro* culture

Stem cells were discovered from analysis of a type of cancer called a teratocarcinoma. In 1964, researchers noted that a single cell in teratocarcinomas could be isolated and remain undifferentiated in culture. These types of stem cells became known as embryonic carcinoma cells (EC cells).^[2] Researchers learned that primordial embryonic germ cells (EG cells) could be cultured and stimulated to produce many different cell types.

Embryonic stem cells (ES cells) were first derived from mouse embryos in 1981 by Martin Evans and Matthew Kaufman and independently by Gail R. Martin. Gail R. Martin is credited with coining the term 'Embryonic Stem Cell'.^{[3][4]} A breakthrough in human embryonic stem cell research came in November 1998 when a group led by James Thomson at the University of Wisconsin-Madison first developed a technique to isolate and grow the cells when derived from human blastocysts.^[5]

Contamination by reagents used in cell culture

The online edition of *Nature Medicine* published a study on January 24, 2005 which stated that the human embryonic stem cells available for federally funded research are contaminated with non-human molecules from the culture medium used to grow the cells.^[6] It is a common technique to use mouse cells and other animal cells to maintain the pluripotency of actively dividing stem cells. The problem was discovered when non-human sialic acid in the growth media was found to compromise the potential uses of the embryonic stem cells in humans, according to scientists at the University of California, San Diego.^[7]

However, a study published in the online edition of *Lancet Medical Journal* on March 8, 2005 detailed information about a new stem cell line which was derived from human embryos under completely cell- and serum-free conditions. After more than 6 months of undifferentiated proliferation, these cells demonstrated the potential to form derivatives of all three embryonic germ layers both *in vitro* and in teratomas. These properties were also successfully maintained (for more than 30 passages) with the established stem cell lines.^[8]

Reducing donor-host rejection

There is also ongoing research to reduce the potential for rejection of the differentiated cells derived from ES cells once researchers are capable of creating an approved therapy from ES cell research. One of the possibilities to prevent rejection is by creating embryonic stem cells that are genetically identical to the patient via therapeutic cloning.

An alternative solution for rejection by the patient to therapies derived from non-cloned ES cells is to derive many well-characterized ES cell lines from different genetic backgrounds and use the cell line that is most similar to the patient; treatment can then be tailored to the patient, minimizing the risk of rejection.

Therapeutic application

On January 23, 2009, Phase I clinical trials for transplantation of a human-ES-derived cell population into spinal cord-injured individuals received FDA approval, marking it the world's first human ES cell human trial ^[9]. The study leading to this scientific advancement was conducted by Hans Keirstead and colleagues at the University of California, Irvine and supported by Geron Corporation of Menlo Park, CA. The results of this experiment suggested an improvement in locomotor recovery in spinal cord-injured rats after a 7-day delayed transplantation of human ES cells that were pushed towards an oligodendrocytic lineage ^[10].

Potential method for new cell line derivation

See also: Induced pluripotent stem cell

On August 23, 2006, the online edition of *Nature* scientific journal published a letter by Dr. Robert Lanza (medical director of Advanced Cell Technology in Worcester, MA) stating that his team had found a way to extract embryonic stem cells without destroying the actual embryo. ^[11] This technical achievement would potentially enable scientists to work with new lines of embryonic stem cells derived using public funding in the USA, where federal funding was at the time limited to research using embryonic stem cell lines derived prior to August 2001. In March, 2009, the limitation was lifted. ^[12]

Professor Shinya Yamanaka had a recent breakthrough ^[13] in which the skin cells of laboratory mice were genetically manipulated back to their embryonic state. This work was confirmed by two other groups, demonstrating that the addition of just 4 genes (Oct3/4, Sox2, Klf4, and c-Myc) could reprogram mouse skin cells into embryonic stem like cells. The ability to reproduce such findings are very important in science and the stem cell field, especially after Hwang Woo-Suk from Korea fabricated data, claiming to have generated human ES cells from cloned embryos. These cells produced by Yamanaka as well as the other laboratories demonstrated all the hallmarks of embryonic stem cells including the ability to form chimeric mice and contribute to the germ-line. One issue with this work is that the mice generated from these ES lines were prone to develop cancer due to the use of Myc, which is a known oncogene.

On the 20th of November, 2007, two research teams, one of which was headed by Professor Yamanaka and the other by James Thomson ^[14] announced a similar breakthrough with ordinary human skin cells that were transformed into batches of cells that look and act like embryonic stem cells. This may enable the generation of patient specific ES cell lines that could potentially be used for cell replacement therapies. In addition, this will allow the generation of ES cell lines from patients with a variety of genetic diseases and will provide invaluable models to study those diseases.

There is still much work to be done before this technology can be used for the treatments of disease. First, the genes used to reprogram the skin cells into ES-like cells were added by the use of retroviruses that can cause mutations and lead to the risk of possible cancers, although recent research by professor Yamanaka's research group has made advances in avoiding this particular problem. ^[15]

In addition, as shown with the mouse work, one of the genes used to reprogram, Myc, can also cause cancer. The group led by Thomson did not use Myc to reprogram and may not have this difficulty. Future work is aimed at attempting to reprogram without permanent genetic manipulation of the cells with viruses. This could be accomplished by either small molecules or other methodologies to express

these reprogramming genes.

However, as a first indication that the induced pluripotent stem (iPS) cell technology can in rapid succession lead to new cures, it was used by a research team headed by Rudolf Jaenisch of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, to cure mice of sickle cell anemia, as reported by Science journal's online edition on 6th of December.^[16]

On January 16, 2008, a California based company, Stemagen, announced that they had created the first mature cloned human embryos from single skin cells taken from adults. These embryos can be harvested for patient matching embryonic stem cells.^[17]

Use of Human Embryonic Stem Cells as Models for Human Genetic Disorders

In recent years there have been several reports regarding the potential use of human embryonic stem cells as models for human genetic diseases. This issue is especially important due to the species-specific nature of many genetic disorders. The relative inaccessibility of human primary tissue for research is another major hindrance. Several new studies have started to address this issue. This has been done either by genetically manipulating the cells, or more recently by deriving diseased cell lines identified by prenatal genetic diagnosis (PGD). This approach, although not directly leading to therapy, may very well prove invaluable at studying disorders such as Fragile-X syndrome, Cystic-Fibrosis, and other genetic maladies that have no reliable model system.

Embryonic Stem Cell Trial Approved By The FDA

This summer, the first ever clinical trial involving embryonic stem cell use on humans has been approved by the FDA. A biotech company called The Geron Corporation will be conducting the trial. The study will "inject neural stem cells into patients suffering from spinal cord injuries" (Strickland). The trial is directed specifically toward paraplegics who have had recent spinal cord injuries. About eight to ten paraplegics who have had their injuries no longer than two weeks before the trial begins, will be selected to take part in the trial as implants must be implemented before scar tissue is able to form. From there, researchers are emphasizing that the injections are not expected to fully cure the patients and restore all mobility. Based on the results of the mice trials, researchers say restoration of myelin sheaths, and an increase in some mobility is highly possible. This first trial is mainly testing the safety of these procedures and if everything goes well, it could lead to future studies "involving more severely disabled patients and larger injections may follow" (Strickland). This could be profound for those with cancer, vision loss, burns, Diabetes, Multiple Sclerosis, Parkinson's disease, Alzheimer's disease, and other degenerative diseases. "ReNeuron, another biotech company received the go ahead from British regulators to test an embryonic stem cell based therapy on a dozen stroke patients in Scotland. The trial, which would inject neural stem cells into the brains of stroke victims, is also set to begin this summer" (Strickland). Reference:<http://blogs.discovermagazine.com/80beats/2009/01/23/fda-approves-the-first-clinical-trials-using-embryonic-stem-cells/>

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See also

- Embryonic Stem Cell Research Oversight (ESCRO) Committees
- Embryoid body

- Stem cell controversy

External links

- Understanding Stem Cells: A View of the Science and Issues from the National Academies (<http://dels.nas.edu/bis/stemcells/booklet.shtml>)
- National Institutes of Health (<http://stemcells.nih.gov/>)

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Germ cell

From Wikipedia, the free encyclopedia

Germ cells are progenitors of the gametes. These singled-out cells move through the gut to the developing gonads and undergo mitotic proliferation followed by meiosis and differentiation into either eggs or sperm (mature gametes). Plants do not have a germ line set aside in early development. Instead germ cells can come from somatic cells in the adult floral meristem.^{[1] [2][3]}

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- 2 Specification
- 3 Migration
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Introduction

Multicellular eukaryotes are made of two fundamental cell types. Germ cells produce gametes and are the only cells that can undergo meiosis as well as mitosis. These cells are often considered as immortal because they are the link between generations. Somatic cells are all the other cells that form the building blocks of the body and they only divide by mitosis. The lineage of germ cells is called germ line. Germ cell specification begins during cleavage in many animals or in the epiblast during gastrulation in birds and mammals. After transport, involving passive movements and active migration, germ cells arrive at the developing gonads. In humans, sexual differentiation starts approximately 6 weeks after conception. The end-products of the germ cell cycle are the egg or sperm.^[4]

Under special conditions *in vitro* germ cells can acquire properties similar to those of embryonic stem cells (ES). The underlying mechanism of that change is still unknown. These changed cells are then called embryonic germ cells (EG). Both EG and ES are pluripotent. Recent studies have demonstrated that it is possible to give rise to primordial germ cells from ES.^[5]

Specification

There are two mechanisms to establish the germ cell lineage in the embryo. The first way is called preformistic and involves that the cells destined to become germ cells inherit the specific germ cell determinants present in the germ plasm (specific area of the cytoplasm) of the egg (ovum). The

unfertilized egg of most animals is asymmetrical: different regions of cytoplasm contain different amounts mRNA and proteins. By this germ cells obtained by the first divisions of the fertilized egg are characterized by specific molecules of a particular region of the egg cytoplasm. The second way is found in birds and mammals, where germ cells are not specified by such determinants but by signals controlled by zygotic genes. In mammals, a few cells of the early embryo are induced by signals of neighboring cells to become primordial germ cells. Mammalian eggs are kind of symmetrical and after the first divisions of the fertilized egg, the produced cells are all totipotent. This means that they can differentiate in any cell type in the body and thus germ cells.

Migration

Primordial germ cells, germ cells that still have to reach the gonads, also known as PGCs, precursor germ cells or gonocytes, divide repeatedly on their migratory route through the gut and into the developing gonads.

Invertebrates

In the model organism *Drosophila*, pole cells passively move from the posterior end of the embryo to the posterior midgut because of the infolding of the blastoderm. Then they actively move through the gut into the mesoderm. Endodermal cells differentiate and together with Wunen proteins they induce the migration through the gut. Wunen proteins are chemorepellants that lead the germ cells away from the endoderm and into the mesoderm. After splitting into two populations, the germ cells continue migrating laterally and in parallel until they reach the gonads. Columbus proteins, chemoattractants, stimulate the migration in the gonadal mesoderm.

Vertebrates

In the *Xenopus* egg, the germ cell determinants are found in the most vegetal blastomeres. These presumptive PGCs are brought to the endoderm of the blastocoel by gastrulation. They are determined as germ cells when gastrulation is completed. Migration from the hindgut along the gut and across the dorsal mesentery then takes place. The germ cells split into two populations and move to the paired gonadal ridges. Migration starts with 3-4 cells that undergo three rounds of cell division so that about 30 PGCs arrive at the gonads. On the migratory path of the PGCs, the orientation of underlying cells and their secreted molecules such as fibronectin play an important role.

Mammals have a migratory path comparable to that in *Xenopus*. Migration begins with 50 gonocytes and about 5,000 PGCs arrive at the gonads. Proliferation occurs also during migration and lasts for 3-4 weeks in humans. PGCs come from the epiblast and migrate subsequently into the mesoderm, the endoderm and the posterior of the yolk sac. Migration then takes place from the hindgut along the gut and across the dorsal mesentery to reach the gonads (4.5 weeks in human beings). Fibronectin maps here also a polarized network together with other molecules. The somatic cells on the path of germ cells provide them attractive, repulsive, and survival signals. But germ cells also send signals to each other.

In reptiles and birds, germ cells use another path. PGCs come from the epiblast and move to the hypoblast to form the germinal crescent (anterior extraembryonic structure). The gonocytes then squeeze into blood vessels and use the circulatory system for transport. They squeeze out of the vessels when they are at height of the gonadal ridges. Cell adhesion on the endothelium of the blood vessels and molecules such as chemoattractants are probably involved in helping PGCs migrate.

Sex determining region of Y (Sry) gene

The sex of a mammalian individual is determined by the *Sry* gene on the Y chromosome. It induces the somatic cells of the gonadal ridge to develop into a testis. *Sry* is expressed in a small group of somatic cells of the developing gonad and influence these cells to become Sertoli cells (supporting cells in testis). Sertoli cells are responsible for sexual development along a male pathway in many ways. One of these ways involves stimulation of the arriving primordial cells to differentiate into sperm. In the absence of the *Sry* gene, primordial germ cells differentiate into eggs. Removing genital ridges before they started to develop into testes or ovaries results in the development of a female, independent of the carried sex chromosome.

Gametogenesis

Gametogenesis, the development of diploid germ cells into either haploid eggs or sperm, (respectively oogenesis and spermatogenesis) is different for each species but the general stages are similar. Oogenesis and spermatogenesis have many features in common, they both involve:

- Meiosis
- Extensive morphological differentiation
- Incapacity of surviving for very long if fertilization does not occur

Despite their homologies they also have major differences:

- Spermatogenesis has equivalent meiotic divisions resulting in four equivalent spermatids while oogenic meiosis is asymmetrical: only one egg is formed together with two polar bodies.
- Different timing of maturation: oogenesis is interrupted at one or more stages (for a long time) while spermatogenic meiosis is rapid and uninterrupted.

Oogenesis

After migration primordial germ cells will become oogonia in the forming gonad (ovary). The oogonia proliferate extensively by mitotic divisions, up to 5-7 million cells in humans. But then many of these oogonia die and about 50,000 remain. These cells differentiate into primary oocytes. In week 11-12 *post coitus* the first meiotic division begins (before birth for most mammals) and remains arrested in prophase I from a few days to many years depending on the species. It is in this period or in some cases at the beginning of sexual maturity that the primary oocytes secrete proteins to form a coat called zona pellucida and they also produce cortical granules containing enzymes and proteins needed for fertilization. Meiosis stands by because of the follicular granulosa cells that send inhibitory signals through gap junctions and the zona pellucida. Sexual maturation is the beginning of periodic ovulation. Ovulation is the regular release of one oocyte from the ovary into the reproductive tract and is preceded by follicular growth. A few follicle cells are stimulated to grow but only one oocyte is ovulated. A primordial follicle consists of an epithelial layer of follicular granulosa cells enclosing an oocyte. The pituitary gland secretes follicle-stimulating hormones (FSHs) that stimulate follicular growth and oocyte maturation. The thecal cells around each follicle secrete estrogen. This hormone stimulates the production of FSH receptors on the follicular granulosa cells and has at the same time a negative feedback on FSH secretion. This results in a competition between the follicles and only the follicle with the most FSH receptors survives and is ovulated. Meiotic division I goes on in the ovulated oocyte stimulated by luteinizing hormones (LHs) produced by the pituitary gland. FSH and LH block the gap junctions between follicle cells and the oocyte therefore inhibiting communication between them. Most

follicular granulosa cells stay around the oocyte and so form the cumulus layer. Large non-mammalian oocytes accumulate egg yolk, glycogen, lipids, ribosomes, and the mRNA needed for protein synthesis during early embryonic growth. These intensive RNA biosyntheses are mirrored in the structure of the chromosomes, which decondense and form lateral loops giving them a lampbrush appearance. Oocyte maturation is the following phase of oocyte development. It occurs at sexual maturity when hormones stimulate the oocyte to complete meiotic division I. The meiotic division I produces 2 cells differing in size: a small polar body and a large secondary oocyte. The secondary oocyte undergoes meiotic division II and that results in the formation of a second small polar body and a large mature egg, both being haploid cells. The polar bodies degenerate.^[6] Oocyte maturation stands by at metaphase II in most vertebrates. During ovulation, the arrested secondary oocyte leaves the ovary and matures rapidly into an egg ready for fertilization. Fertilization will cause the egg to complete meiosis II. In human females there is proliferation of the oogonia in the fetus, meiosis starts then before birth and stands by at meiotic division I up to 50 years, ovulation begins at puberty.

Egg growth

A 10 - 20 μm large somatic cell generally needs 24 hours to double its mass for mitosis. By this way it would take a very long time for that cell to reach the size of a mammalian egg with a diameter of 100 μm (some insects have eggs of about 1,000 μm or greater). Eggs have therefore special mechanisms to grow to their large size. One of these mechanisms is to have extra copies of genes: meiotic division I is paused so that the oocyte grows while it contains two diploid chromosome sets. Some species produce many extra copies of genes, such as amphibians, which may have up to 1 or 2 million copies. A complementary mechanism is partly dependent on syntheses of other cells. In amphibians, birds, and insects, yolk is made by the liver (or its equivalent) and secreted into the blood. Neighboring accessory cells in the ovary can also provide nutritive help of two types. In some invertebrates some oogonia become nurse cells. These cells are connected by cytoplasmic bridges with oocytes. The nurse cells of insects provide oocytes macromolecules such as proteins and mRNA. Follicular granulosa cells are the second type of accessory cells in the ovary in both invertebrates and vertebrates. They form a layer around the oocyte and nourish them with small molecules, no macromolecules, but eventually their smaller precursor molecules, by gap junctions.

Spermatogenesis

Mammalian spermatogenesis is representative for most animals. In human males, spermatogenesis begins at puberty in seminiferous tubules in the testes and go on continuously. Spermatogonia are immature germ cells. They proliferate continuously by mitotic divisions around the outer edge of the seminiferous tubules, next to the basal lamina. Some of these cells stop proliferation and differentiate into primary spermatocytes. After they proceed through the first meiotic division, two secondary spermatocytes are produced. The two secondary spermatocytes undergo the second meiotic division to form four haploid spermatids. These spermatids differentiate morphologically into sperm by nuclear condensation, ejection of the cytoplasm and formation of the acrosome and flagellum.

The developing male germ cells do not complete cytokinesis during spermatogenesis. Consequently cytoplasmic bridges assure connection between the clones of differentiating daughter cells to form a syncytium. In this way the haploid cells are supplied with all the products of a complete diploid genome. Sperm that carry a Y chromosome, for example, is supplied with essential molecules that are encoded by genes on the X chromosome.

Diseases

Germ cell tumor is a rare cancer that can affect people at all ages. 2.4 children out of 1 million suffer the disease, and it counts for 4% of all cancers in children and adolescents younger than 20 years old. Germ cell tumors are generally located in the gonads but can also appear in the abdomen, pelvis, mediastinum, or brain. Germ cells migrating to the gonads may not reach that intended destination and a tumor can grow wherever they end up, but the exact cause is still unknown. These tumors can be benign or malignant.^[7]

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External links

- MeSH *Germ+Cells* (http://www.nlm.nih.gov/cgi/mesh/2009/MB_cgi?mode=&term=Germ+Cells)

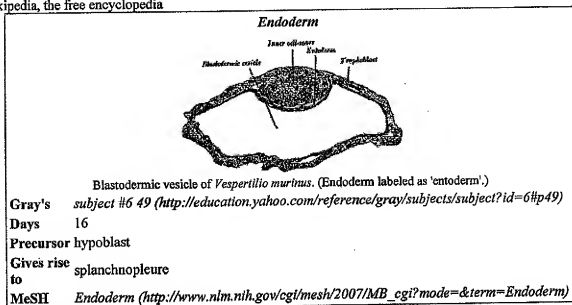
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Endoderm

From Wikipedia, the free encyclopedia



Endoderm, (sometimes called *Entoderm*) is one of the germ layers formed during animal embryogenesis. Cells migrating inward along the archenteron from the inner layer of the gastrula, which develops into the endoderm.

The endoderm consists at first of flattened cells, which subsequently become columnar. It forms the epithelial lining of the whole of the digestive tube except part of the mouth, pharynx and the terminal part of the rectum (which are lined by involutions of the ectoderm), the lining cells of all the glands which open into the digestive tube, including those of the liver and pancreas, the epithelium of the auditory tube and tympanic cavity, of the trachea, bronchi, and alveoli of the lungs, of the urinary bladder and part of the urethra, and that which lines the follicles of the thyroid gland and thymus.

Contents

- 1 Production
- 2 Additional images
- 3 See also
- 4 Notes
- 5 References

Production

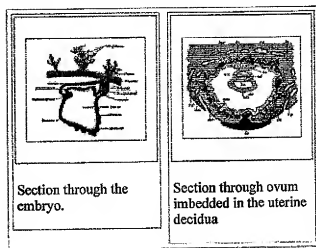
The following chart shows the products produced by the endoderm.

Germ Layer	Category	Product
Endoderm	General ^[1]	Gastrointestinal tract
Endoderm	General	Respiratory tract

Germ Layer	Category	Product
Endoderm	General ^[1]	Gastrointestinal tract
Endoderm	General	Respiratory tract
Endoderm	General	Endocrine glands and organs (liver and pancreas)

The Endoderm has differentiated into distinguishable organs as early as 5 weeks of embryonic development.

Additional images



See also

- Ectoderm
- Germ layer
- Histogenesis
- Mesoderm
- Organogenesis
- Endodermal sinus tumor

Notes

- ¹ ^ The **General** category denotes that all or most of the animals containing this layer produce the adjacent product.

References

- Evers, Christine A., Lisa Starr. *Biology: Concepts and Applications*. 6th ed. United States:Thomson, 2006. ISBN 0-534-46224-3.

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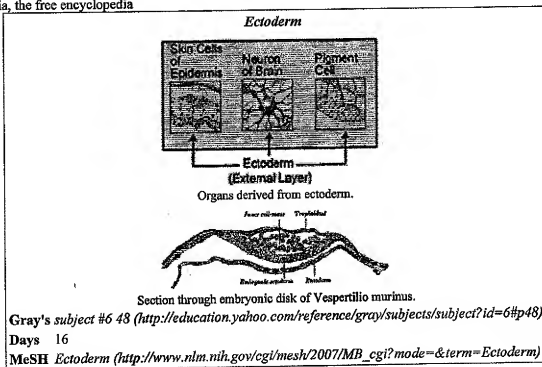
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Ectoderm

From Wikipedia, the free encyclopedia

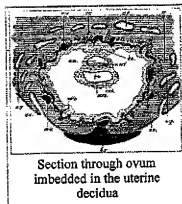
The **ectoderm** is the start of a tissue that covers the body surfaces. It emerges first and forms from the outermost of the germ layers.

Generally speaking, the ectoderm



differentiates to form the nervous system, and the epidermis (the outer part of integument).

In vertebrates, the ectoderm has three parts: external ectoderm (also known as surface ectoderm), the neural crest, and neural tube. The latter two are known as neuroectoderm.



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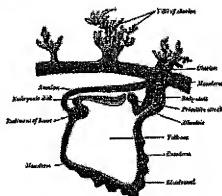
Mesoderm

From Wikipedia, the free encyclopedia

One of the three germ layers found in the embryos of animals more complex than



Organs derived from mesoderm.



Section through the embryo

Gray's subject #6 49 (<http://education.yahoo.com/reference/gray/subjects/subject?id=6#p49>)

Days 16

MeSH Mesoderm (http://www.nlm.nih.gov/cgi/mesh/2007/MB_cgi?mode=&term=Mesoderm)

cnidarians, making them triploblastic. Mesoderm forms in the embryo during gastrulation when some of the cells migrating inward to form the endoderm, produce an additional layer that lies between the endoderm and the ectoderm.

Mesoderm is found in all large, complex animals, and allows the formation of a coelom, which allows more room for independent growth of the body organs.

Contents

- 1 Mesoderm Derivatives
 - 1.1 General
 - 1.2 Vertebrates
- 2 See also
- 3 References
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Mesoderm Derivatives

General

The body organs, tissues and systems derived from the mesoderm in most triploblastic animals can be listed as follows:

- bones
- cartilage
- most of the circulatory system, including the heart and major blood vessels
- connective tissues of the gut and integuments
- mesenchyme
- mesothelium
- muscles
- peritoneum (lining of the abdominal cavity)
- reproductive system
- spleen
- urinary system, including the kidneys

Vertebrates

Before formation of the items in the above list, the mesoderm of a developing vertebrate transitionally differentiates into the following sub-types:

- Chordamesoderm (also known as *axial mesoderm*) which later on gives rise to notochord in all chordates
- Paraxial mesoderm
- Intermediate mesoderm
- Lateral plate mesoderm

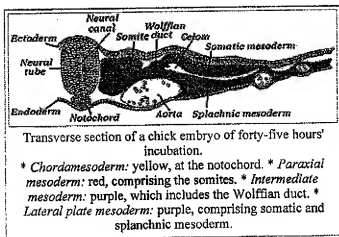
See also

- Embryo
- Embryogenesis
- Embryology
- Ectoderm
- Endoderm
- Germ layer
- Histogenesis
- Organogenesis

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- Evers, Christine A., Lisa Starr. *Biology: Concepts and Applications*. 6th ed. United States: Thomson, 2006. ISBN 0-534-46224-3.

External links



- Embryology at UNSW *Notes/skmus6* (<http://embryology.med.unsw.edu.au/Notes/skmus6.htm>)
- Embryology at Temple *EMBIII97/sld039*
(<http://isc.temple.edu/marino/embryology/EMBIII97/sld039.htm>)

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Appendix B

MOLECULAR BIOLOGY OF
THE CELL

fourth edition

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Alexander Johnson

Julian Lewis

Martin Raff

Keith Roberts

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somite

One of a series of paired blocks of mesoderm that form during early development and lie on either side of the notochord in a vertebrate embryo. They give rise to the vertebral column, muscles and associated connective tissue. Each somite produces the musculature of one vertebral segment, plus associated connective tissue.

sorting signal

Amino acid sequence that directs the delivery of a protein to a specific location outside the cytosol.

Southern blotting

Technique in which DNA fragments separated by electrophoresis are immobilized on a paper sheet. Specific fragments are then detected with a labeled nucleic acid probe. (Named after E.M. Southern, inventor of the technique.)

spectrin

Abundant protein associated with the cytosolic side of the plasma membrane in red blood cells, forming a rigid network that supports the membrane.

Spemann's Organizer

Specialized tissue at the dorsal lip of the blastopore in an amphibian embryo; a source of signals that help to orchestrate formation of the embryonic body axis. (After H. Spemann and H. Mangold, co-discoverers.)

sperm (spermatozoon, plural spermatozoa)

The mature male gamete in animals. It is motile and usually small compared with the egg.

spermatogenesis

Development of sperm.

spindle-attachment checkpoint

Checkpoint that operates during mitosis to ensure that all chromosomes are properly attached to the spindle before sister-chromatid separation starts.

spliceosome

Large assembly of RNA and protein molecules that performs pre-mRNA splicing in eucaryotic cells.

Src family

Family of cytoplasmic tyrosine kinases (pronounced "sark") that associate with the cytoplasmic domains of some enzyme-linked receptors (for example, the T cell antigen receptor) that lack intrinsic tyrosine kinase activity. They transmit a signal onwards by phosphorylating the receptor itself and other signaling proteins.

SRP—see signal-recognition particle**standard free-energy change (ΔG°)**

Free-energy change of two reacting molecules at standard temperature and pressure when all components are present at a concentration of 1 mole per liter.

starch

Polysaccharide composed exclusively of glucose units, used as an energy storage material in plant cells.

start-transfer signal

Short amino-acid sequence that enables a polypeptide chain to start being translocated across the endoplasmic reticulum membrane through a protein translocator. Multipass membrane proteins have both N-terminal (signal sequence) and internal start-transfer signals.

stem cell

Relatively undifferentiated cell that can continue dividing indefinitely, throwing off daughter cells that can undergo terminal differentiation into particular cell types.

stereocilium

A large, rigid microvillus found in "organ pipe" arrays on the apical surface of hair cells in the ear. A stereocilium contains a bundle of actin filaments, rather than microtubules, and is thus not a true cilium.

steroid

Hydrophobic lipid molecule with a characteristic four-ringed structure. Many important hormones such as estrogen and testosterone are steroids. (See Panel 2-5, pp. 118-119.)

stimulatory G protein (G_s)

G protein that, when activated, activates the enzyme adenylyl cyclase and thus stimulates the production of cyclic AMP.

stop-transfer signal

Hydrophobic amino acid sequence that halts translocation of a polypeptide chain through the endoplasmic reticulum membrane, thus anchoring the protein chain in the membrane. (See Figure 12-49.)

strand-directed mismatch repair—see mismatch repair**striated muscle**

Muscle composed of transversely striped (striated) myofibrils. Skeletal and heart muscle of vertebrates are the best-known examples.

stroma

(1) The connective tissue in which a glandular or other epithelium is embedded. (2) The large interior space of a chloroplast, containing enzymes that incorporate CO_2 into sugars.

structural gene

Region of DNA that codes for a protein or for an RNA molecule that forms part of a structure or has an enzymatic function. Distinguished from regions of DNA that regulate gene expression.

substrate

Molecule on which an enzyme acts.

substratum

Solid surface to which a cell adheres.

subunit

Component of a multicomponent complex—for example, one protein component of a protein complex or one polypeptide chain of a multichain protein.

sucrose

Disaccharide composed of one glucose unit and one fructose unit. The major form in which glucose is transported between plant cells.

sugar

Small carbohydrates with a monomer unit of general formula $(CH_2O)_n$. Examples are the monosaccharides glucose, fructose and mannose, and the disaccharide sucrose (composed of a molecule of glucose and one of fructose linked together).

sulfhydryl (thiol, -SH)

Chemical group containing sulfur and hydrogen found in the amino acid cysteine and other molecules. Two sulfhydryl can join to produce a disulfide bond.

supercoiled DNA

Region of DNA in which the double helix is further twisted on itself. (See Figure 6-20.)

survival factor

Extracellular signal required for a cell to survive; in its absence the cell will undergo apoptosis and die.

dynamic instability

The property of sudden conversion from growth to shrinkage, and vice versa, in a protein filament such as a microtubule or actin filament. (See Panel 18-2, pp. 912-913.)

dynamin

Cytosolic GTPase that binds to the neck of a clathrin-coated vesicle in the process of budding from the membrane, and which is involved in completing vesicle formation.

dynema

Member of a family of large motor proteins that undergo ATP-dependent movement along microtubules. In cilia, dynein forms the side arms in the axoneme that cause adjacent microtubule doublets to slide past one another.

dysplasia

A change in cell growth and behavior in a tissue in which the structure becomes disordered.

ectoderm

Embryonic tissue that is the precursor of the epidermis and nervous system.

effector cell

A cell that carries out the final response or function of a particular process. The main effector cells of the immune system, for example, are activated lymphocytes and phagocytes—the cells involved in destroying pathogens and removing them from the body.

egg

The mature female gamete in sexually reproducing organisms. It is usually a large and immobile cell.

elastin

Hydrophobic protein that forms extracellular extensible fibres (elastic fibres) that give tissues their stretchability and resilience.

electrochemical gradient

The combined influence of a difference in the concentration of an ion on the two sides of the membrane and the electrical charge difference across the membrane (membrane potential). It produces a driving force that causes the ion to move across the membrane.

electrochemical proton gradient

The result of a combined pH gradient (proton gradient) and the membrane potential.

electron

Negatively charged subatomic particle that generally occupies orbitals surrounding the nucleus in an atom.

electron acceptor

Atom or molecule that takes up electrons readily, thereby gaining an electron and becoming reduced.

electron carrier

Molecule such as cytochrome c, which transfers an electron from a donor molecule to an acceptor molecule.

electron donor

Molecule that easily gives up an electron, becoming oxidized in the process.

electron microscope

Type of microscope that uses a beam of electrons to create the image.

electron-transport chain

Series of electron carrier molecules along which electrons move from a higher to a lower energy level to a final acceptor molecule. The energy released during electron movement can be used to power various processes. Electron-transport chains present in the inner mitochondrial membrane and in the thylakoid membrane of chloroplasts generate a proton

gradient across the membrane that is used to drive ATP synthesis.

elongation factor

Protein required for the addition of amino acids to growing polypeptide chains on ribosomes.

embryogenesis

Development of an embryo from a fertilized egg, or zygote.

embryonic stem cell (ES cell)

Cell derived from the inner cell mass of the early mammalian embryo that can give rise to all the cells in the body. It can be grown in culture, genetically modified and inserted into a blastocyst to develop a transgenic animal.

endocrine cell

Specialized animal cell that secretes a hormone into the blood. Usually part of a gland, such as the thyroid or pituitary gland.

endocytic-exocytic cycle

The processes of endocytosis and exocytosis that, respectively, add and remove plasma membrane from the cell, resulting in no overall change in the cell's surface area and volume.

endocytosis

Uptake of material into a cell by an invagination of the plasma membrane and its internalization in a membrane-bounded vesicle. (See also pinocytosis and phagocytosis.)

endoderm

Embryonic tissue that is the precursor of the gut and associated organs.

endoplasmic reticulum (ER)

Labyrinthine membrane-bounded compartment in the cytoplasm of eucaryotic cells, where lipids are synthesized and membrane-bound proteins and secretory proteins are made.

endosome

Membrane-bounded organelle in animal cells that carries materials newly ingested by endocytosis and passes many of them on to lysosomes for degradation.

endothelial cell

Flattened cell type that forms a sheet (the endothelium) lining all blood vessels.

enhancer

Regulatory DNA sequence to which gene regulatory proteins bind, influencing the rate of transcription of a structural gene that can be many thousands of base pairs away.

entropy

Thermodynamic quantity that measures the degree of disorder in a system; the higher the entropy, the greater the disorder.

enveloped virus

Virus with a capsid surrounded by a lipid membrane (the envelope), which is derived from the host cell plasma membrane when the virus buds from the cell.

enzyme

Protein that catalyzes a specific chemical reaction.

enzyme-linked receptor

Major type of cell-surface receptor in which the cytoplasmic domain either has enzymatic activity itself or is associated with an intracellular enzyme. In both cases enzymatic activity is stimulated by ligand binding to the receptor.

epidermis

Epithelial layer covering the outer surface of the body. It has different structures in different animal groups. The outer layer of plant tissue is also called the epidermis.

epimerization

Reaction that alters the steric arrangement around one atom, as in a sugar molecule.

epinephrine—see **adrenaline**

epithelial tissue—see **epithelium**

epithelium (plural **epithelia**)

Coherent cell sheet formed from one or more layers of cells covering an external surface or lining a cavity.

epitope—see **antigenic determinant**

equilibrium constant (*K*)

Ratio of forward and reverse rate constants for a reaction and equal to the association constant. (See Figure 3-44.)

equilibrium

State where there is no net change in a system. For example, equilibrium is reached in a chemical reaction when the forward and reverse rates are equal.

ER lumen

The space enclosed by the membrane of the endoplasmic reticulum (ER).

ER resident protein

Protein that remains in the endoplasmic reticulum (ER) or its membranes and carries out its function there, as opposed to proteins that are present in the ER only in transit.

ER retention signal

Short amino acid sequence on a protein that prevents it moving out of the endoplasmic reticulum (ER). Found on proteins that are resident in the ER and function there.

ER signal sequence

N-terminal signal sequence that directs proteins to enter the endoplasmic reticulum (ER). It is cleaved off by signal peptidase after entry.

ER—see **endoplasmic reticulum**

erythrocyte (red blood cell)

Small, hemoglobin-containing blood cell of vertebrates that transports oxygen and carbon dioxide to and from tissues.

erythropoietin

Growth factor that stimulates the production of red blood cells. It is produced by the kidney and acts on precursor cells in bone marrow.

ES cell—see **embryonic stem cell**

***Escherichia coli* (*E. coli*)**

Rodlike bacterium normally found in the colon of humans and other mammals and widely used in biomedical research.

ester

Molecule formed by the condensation reaction of an alcohol group with an acidic group. Phosphate groups usually form esters when linked to a second molecule. (See Panel 2-1, 110-111.)

ethyl ($-\text{CH}_2\text{CH}_3$)

Hydrophobic chemical group derived from ethane, (CH_3CH_3) .

eucaryote (eukaryote)

Organism composed of one or more cells with a distinct nucleus and cytoplasm. Includes all forms of life except viruses and prokaryotes (bacteria and archaea).

euchromatin

Region of an interphase chromosome that stains diffusely; "normal" chromatin, as opposed to the more condensed heterochromatin.

exocytosis

Process by which most molecules are secreted from a eucaryotic cell. These molecules are packaged in membrane-bounded vesicles that fuse with the plasma membrane, releasing their contents to the outside.

exon

Segment of a eucaryotic gene that consists of a sequence of nucleotides that will be represented in messenger RNA or the final transfer RNA or ribosomal RNA. In protein-coding genes, exons encode amino acids in the protein. An exon is usually adjacent to a noncoding DNA segment called an intron.

expression vector

A virus or plasmid that carries a DNA sequence into a suitable host cell and there directs the synthesis of the protein encoded by the sequence.

expression

Production of an observable phenotype by a gene—usually by directing the synthesis of a protein.

extracellular matrix

Complex network of polysaccharides (such as glycosaminoglycans or cellulose) and proteins (such as collagen) secreted by cells. Serves as a structural element in tissues and also influences their development and physiology.

facilitated diffusion—see **passive transport**

FADH₂ (reduced flavin adenine dinucleotide)

Activated carrier molecule that is produced by the citric acid cycle.

FAK—see **focal adhesion kinase**

Fas protein (Fas)

Membrane-bound receptor that initiates apoptosis in the receptor-bearing cell after binding to its ligand (Fas ligand).

fat

Energy-storage lipid in cells. It is composed of triglycerides—fatty acids esterified with glycerol.

fat cell

Connective-tissue cell that produces and stores fat in animals.

fatty acid

Compound such as palmitic acid that has a carboxylic acid attached to a long hydrocarbon chain. Used as a major source of energy during metabolism and as a starting point for the synthesis of phospholipids. (See Panel 2-5, pp. 118-119.)

Fc receptor

One of a family of receptors specific for the invariant constant region (Fc region) of immunoglobulins (other than IgM and IgD); different Fc receptors are specific for IgG, IgA, IgE and their subclasses.

feedback inhibition

Type of regulation of metabolism in which an enzyme acting early in a reaction pathway is inhibited by a late product of that pathway.

fermentation

Anaerobic energy-yielding metabolic pathway in which pyruvate produced by glycolysis is converted, for example, into lactate or ethanol, with the conversion of NADH to NAD⁺.

fertilization

Fusion of a male and a female gamete (both haploid) to form a diploid zygote, which develops into a new individual.

gap junction

Communicating cell-cell junction that allows ions and small molecules to pass from the cytoplasm of one cell to the cytoplasm of the next.

gastrulation

The stage in animal embryogenesis during which the embryo is transformed from a ball of cells to a structure with a gut (a gastrula).

gene activator protein

A gene regulatory protein that when bound to its regulatory sequence in DNA activates transcription.

gene control region

DNA sequences required to initiate transcription of a given gene and control the rate of initiation.

gene conversion

Process by which DNA sequence information can be transferred from one DNA helix (which remains unchanged) to another DNA helix whose sequence is altered. It occurs occasionally during general recombination.

gene regulatory protein

General name for any protein that binds to a specific DNA sequence to alter the expression of a gene.

gene repressor protein

A gene regulatory protein that prevents the initiation of transcription.

gene

Region of DNA that controls a discrete hereditary characteristic, usually corresponding to a single protein or RNA. This definition includes the entire functional unit, encompassing coding DNA sequences, noncoding regulatory DNA sequences, and introns.

general recombination, general genetic recombination

Recombination that takes place between two homologous chromosomes (as in meiosis).

general transcription factor

Any of the proteins whose assembly around the TATA box is required for the initiation of transcription of most eucaryotic genes.

genetic code

Set of rules specifying the correspondence between nucleotide triplets (codons) in DNA or RNA and amino acids in proteins.

genetic map

Map of the chromosomes in which the distance of genes relative to each other is determined by the amount of genetic recombination that occurs between them.

genetic recombination—see recombination**genetic screen**

A search through a large collection of mutants for a mutant with a particular phenotype.

genome

The totality of genetic information belonging to a cell or an organism; in particular, the DNA that carries this information.

genomic DNA

DNA constituting the genome of a cell or an organism. Often used in contrast to cDNA (DNA prepared by reverse transcription from messenger RNA). Genomic DNA clones represent DNA cloned directly from chromosomal DNA, and a collection of such clones from a given genome is a genomic DNA library.

genomic imprinting

Situation where a gene is either expressed or not expressed in the embryo depending on which parent it is inherited from.

genomics

The science of studying the DNA sequences and properties of entire genomes.

genotype

Genetic constitution of an individual cell or organism.

germ cell

Precursor cell that will give rise to gametes.

germ line

The lineage of germ cells (which contribute to the formation of a new generation of organisms), as distinct from somatic cells (which form the body and leave no descendants).

GFP—see green fluorescent protein**giga-**

Prefix denoting 10^9 . (From Greek *gigas*, giant.)

G_i—see inhibitory G protein**glial cell**

Supporting cell of the nervous system, including oligodendrocytes and astrocytes in the vertebrate central nervous system and Schwann cells in the peripheral nervous system.

globular protein

Any protein with an approximately rounded shape. Such proteins are contrasted with highly elongated, fibrous proteins such as collagen.

glucose

Six-carbon sugar that plays a major role in the metabolism of living cells. Stored in polymeric form as glycogen in animal cells and as starch in plant cells. (See Panel 2-4, pp. 116-117.)

glutaraldehyde

Small reactive molecule with two aldehyde groups that is often used as a cross-linking fixative.

glycerol

Small organic molecule that is the parent compound of many small molecules in the cell, including phospholipids.

glycocalyx (cell coat)

Carbohydrate-rich layer that forms the outer coat of a eucaryotic cell. Composed of the oligosaccharides linked to intrinsic plasma membrane glycoproteins and glycolipids, as well as glycoproteins and proteoglycans that have been secreted and reabsorbed onto the cell surface.

glycogen

Polysaccharide composed exclusively of glucose units used to store energy in animal cells. Large granules of glycogen are especially abundant in liver and muscle cells.

glycolipid

Membrane lipid molecule with a sugar residue or oligosaccharide attached to the polar headgroup. (See Panel 2-5, pp. 118-119.)

glycolysis

Ubiquitous metabolic pathway in the cytosol in which sugars are incompletely degraded with production of ATP. (Literally, "sugar splitting.")

glycoprotein

Any protein with one or more oligosaccharide chains covalently linked to amino-acid side chains. Most secreted proteins and most proteins exposed on the outer surface of the plasma membrane are glycoproteins.

membrane protein

Protein that is normally closely associated with a cell membrane. (See Figure 10-17.)

membrane transport

Movement of molecules across a membrane mediated by a membrane transport protein.

membrane transport protein

Membrane protein that mediates the passage of ions or molecules across a membrane. Examples are ion channels and carrier proteins.

meristem

An organized group of dividing cells whose derivatives give rise to the tissues and organs of a flowering plant. Key examples are the apical meristems at the tips of shoots and roots.

mesenchyme

Immature, unspecialized form of connective tissue in animals, consisting of cells embedded in a thin extracellular matrix.

mesoderm

Embryonic tissue that is the precursor to muscle, connective tissue, skeleton and many of the internal organs.

messenger RNA (mRNA)

RNA molecule that specifies the amino acid sequence of a protein. Produced by RNA splicing (in eucaryotes) from a larger RNA molecule made by RNA polymerase as a complementary copy of DNA. It is translated into protein in a process catalyzed by ribosomes.

metabolism

The sum total of the chemical processes that take place in living cells.

metaphase

Stage of mitosis at which chromosomes are firmly attached to the mitotic spindle at its equator but have not yet segregated toward opposite poles.

metaphase plate

Imaginary plane at right angles to the mitotic spindle and midway between the spindle poles; the plane in which chromosomes are positioned at metaphase.

metaplasia

A change in the pattern of cell differentiation in a tissue.

metastasis

Spread of cancer cells from their site of origin to other sites in the body.

methyl (-CH₃)

Hydrophobic chemical group derived from methane (CH₄).

MHC molecule

One of a large family of ubiquitous cell-surface glycoproteins encoded by genes of the major histocompatibility complex (MHC). They bind peptide fragments of foreign antigens and present them to T cells to induce an immune response. (See also class I MHC molecule, class II MHC molecule.)

MHC—see major histocompatibility complex

micro-

Prefix denoting 10⁻⁶.

microelectrode, micropipette

Piece of fine glass tubing pulled to an even finer tip. Used to penetrate a cell to study its physiology or to inject electric current or molecules.

microfilament—see actin filament

micrograph

Photograph of an image seen through a microscope. May be either a light micrograph or an electron micrograph depending on the type of microscope employed.

microinjection

Injection of molecules into a cell using a micropipette.

micron (μm or micrometer)

Unit of measurement often applied to cells and organelles. Equal to 10⁻⁶ meter or 10⁻⁴ centimeter.

micropipette—see microelectrode**microsome**

Small vesicle that is derived from fragmented endoplasmic reticulum produced when cells are homogenized.

microtubule

Long hollow cylindrical structure composed of the protein tubulin. It is one of the three major classes of filaments of the cytoskeleton. (See Panel 18-1, p. 909.)

microtubule-associated protein (MAP)

Any protein that binds to microtubules and modifies their properties. Many different kinds have been found, including structural proteins, such as MAP-2, and motor proteins, such as dynein.

microtubule-organizing center (MTOC)

Region in a cell, such as a centrosome or a basal body, from which microtubules grow.

microvillus (plural microvilli)

Thin cylindrical membrane-covered projection on the surface of an animal cell containing a core bundle of actin filaments. Present in especially large numbers on the absorptive surface of intestinal epithelial cells.

midbody

Structure formed at the end of cleavage that can persist for some time as a tether between the two daughter cells in animals.

milli-

Prefix denoting 10⁻³.

minus end

The end of a microtubule or actin filament at which the addition of monomers occurs least readily; the "slow-growing" end of the microtubule or actin filament. The minus end of an actin filament is also known as the pointed end. (See Panel 18-2, pp. 912-913.)

mismatch repair

DNA repair process that corrects mismatched nucleotides inserted during DNA replication. A short stretch of newly synthesized DNA including the mismatched nucleotide is removed and replaced with the correct sequence with reference to the template strand.

mitochondrial precursor protein

Mitochondrial protein encoded by a nuclear gene, synthesized in the cytosol, and subsequently transported into mitochondria.

mitochondrion (plural mitochondria)

Membrane-bounded organelle, about the size of a bacterium, that carries out oxidative phosphorylation and produces most of the ATP in eucaryotic cells.

mitogen

An extracellular substance, such as a growth factor, that stimulates cell proliferation.

mitogen-activated protein kinase—see MAP-kinase

somite

One of a series of paired blocks of mesoderm that form during early development and lie on either side of the notochord in a vertebrate embryo. They give rise to the vertebral column, muscles and associated connective tissue. Each somite produces the musculature of one vertebral segment, plus associated connective tissue.

sorting signal

Amino acid sequence that directs the delivery of a protein to a specific location outside the cytosol.

Southern blotting

Technique in which DNA fragments separated by electrophoresis are immobilized on a paper sheet. Specific fragments are then detected with a labeled nucleic acid probe. (Named after E.M. Southern, inventor of the technique.)

spectrin

Abundant protein associated with the cytosolic side of the plasma membrane in red blood cells, forming a rigid network that supports the membrane.

Spemann's Organizer

Specialized tissue at the dorsal lip of the blastopore in an amphibian embryo; a source of signals that help to orchestrate formation of the embryonic body axis. (After H. Spemann and H. Mangold, co-discoverers.)

sperm (spermatozoon, plural spermatozoa)

The mature male gamete in animals. It is motile and usually small compared with the egg.

spermatogenesis

Development of sperm.

spindle-attachment checkpoint

Checkpoint that operates during mitosis to ensure that all chromosomes are properly attached to the spindle before sister-chromatid separation starts.

spliceosome

Large assembly of RNA and protein molecules that performs pre-mRNA splicing in eucaryotic cells.

Src family

Family of cytoplasmic tyrosine kinases (pronounced "sark") that associate with the cytoplasmic domains of some enzyme-linked receptors (for example, the T cell antigen receptor) that lack intrinsic tyrosine kinase activity. They transmit a signal onwards by phosphorylating the receptor itself and other signaling proteins.

SRP—see signal-recognition particle**standard free-energy change (ΔG°)**

Free-energy change of two reacting molecules at standard temperature and pressure when all components are present at a concentration of 1 mole per liter.

starch

Polysaccharide composed exclusively of glucose units, used as an energy storage material in plant cells.

start-transfer signal

Short amino-acid sequence that enables a polypeptide chain to start being translocated across the endoplasmic reticulum membrane through a protein translocator. Multipass membrane proteins have both N-terminal (signal sequence) and internal start-transfer signals.

stem cell

Relatively undifferentiated cell that can continue dividing indefinitely, throwing off daughter cells that can undergo terminal differentiation into particular cell types.

stereocilium

A large, rigid microvillus found in "organ pipe" arrays on the apical surface of hair cells in the ear. A stereocilium contains a bundle of actin filaments, rather than microtubules, and is thus not a true cilium.

steroid

Hydrophobic lipid molecule with a characteristic four-ring structure. Many important hormones such as estrogen and testosterone are steroids. (See Panel 2-5, pp. 118-119.)

stimulatory G protein (G_s)

G protein that, when activated, activates the enzyme adenylyl cyclase and thus stimulates the production of cyclic AMP.

stop-transfer signal

Hydrophobic amino acid sequence that halts translocation of a polypeptide chain through the endoplasmic reticulum membrane, thus anchoring the protein chain in the membrane (See Figure 12-49).

strand-directed mismatch repair—see mismatch repair**striated muscle**

Muscle composed of transversely striped (striated) myofibrils. Skeletal and heart muscle of vertebrates are the best-known examples.

stroma

(1) The connective tissue in which a glandular or other epithelium is embedded. (2) The large interior space of a chloroplast, containing enzymes that incorporate CO_2 into sugars.

structural gene

Region of DNA that codes for a protein or for an RNA molecule that forms part of a structure or has an enzymatic function. Distinguished from regions of DNA that regulate gene expression.

substrate

Molecule on which an enzyme acts.

substratum

Solid surface to which a cell adheres.

subunit

Component of a multicomponent complex—for example, one protein component of a protein complex or one polypeptide chain of a multichain protein.

sucrose

Disaccharide composed of one glucose unit and one fructose unit. The major form in which glucose is transported between plant cells.

sugar

Small carbohydrates with a monomer unit of general formula $(CH_2O)_n$. Examples are the monosaccharides glucose, fructose and mannose, and the disaccharide sucrose (composed of a molecule of glucose and one of fructose linked together).

sulphydryl ($-SH$)

Chemical group containing sulfur and hydrogen found in the amino acid cysteine and other molecules. Two sulphydryls can join to produce a disulfide bond.

supercoiled DNA

Region of DNA in which the double helix is further twisted on itself. (See Figure 8-20.)

survival factor

Extracellular signal required for a cell to survive; in its absence the cell will undergo apoptosis and die.

We begin by reviewing some of the basic general principles of animal development and by introducing the seven animal species that developmental biologists have adopted as their chief model organisms.

Animals Share Some Basic Anatomical Features

The similarities between animal species in the genes that control development reflect the evolution of animals from a common ancestor in which these genes were already present. Although we do not know what it looked like, the common ancestor of worms, molluscs, insects, vertebrates, and other complex animals must have had many differentiated cell types that would be recognizable to us: epidermal cells, for example, forming a protective outer layer; gut cells to absorb nutrients from ingested food; muscle cells to move; neurons and sensory cells to control the movements. The body must have been organized with a sheet of skin covering the exterior, a mouth for feeding and a gut tube to contain and process the food—with muscles, nerves and other tissues arranged in the space between the external sheet of skin and the internal gut tube.

These features are common to almost all animals, and they correspond to a common basic anatomical scheme of development. The egg cell—a giant storehouse of materials—divides, or cleaves, to form many smaller cells. These cells then move together to create an epithelial sheet facing the external medium. Much of this sheet remains external, constituting the **ectoderm**—the precursor of the epidermis and of the nervous system. A part of the sheet becomes tucked into the interior to form **endoderm**—the precursor of the gut and its appendages, such as the stomach and liver. Another group of cells move into the space between ectoderm and endoderm, and form the **mesoderm**—the precursor of muscles, connective tissues, and various other components. This transformation of a simple ball or hollow sphere of cells into a structure with a gut is called **gastrulation** (from the Greek word for a belly), and in one form or another it is an almost universal feature of animal development. Figure 21-3 illustrates the process as it is seen in the sea urchin.

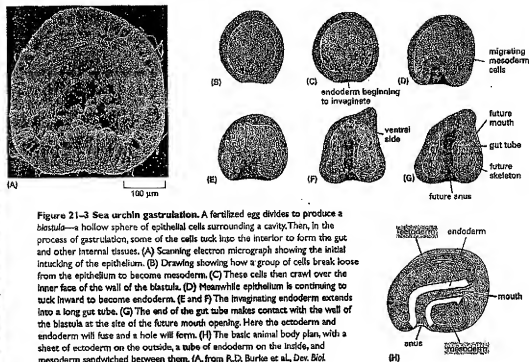


Figure 21-3 Sea urchin gastrulation. A fertilized egg divides to produce a blastula—a hollow sphere of epithelial cells surrounding a cavity. Then, in the process of gastrulation, some of the cells tuck into the interior to form the gut and other internal tissues. (A) Scanning electron micrograph showing the initial tucking of the epithelium. (B) Drawing showing how a group of cells break loose from the epithelium to become mesoderm. (C) These cells then crawl over the inner face of the wall of the blastula. (D) Meanwhile epithelium is continuing to tuck inward to become endoderm. (E and F) The invaginating endoderm extends into a long gut tube. (G) The end of the gut tube makes contact with the wall of the blastula at the site of the future mouth opening. Here the ectoderm and endoderm will fuse and a hole will form. (H) The basic animal body plan, with a sheet of ectoderm on the outside, a tube of endoderm on the inside, and mesoderm sandwiched between them. (A, from R.D. Burke et al., *Dev. Biol.* 146:542–557, 1991. © Academic Press; B–G, after L. Wolpert and T. Gustafson, *Endeavour* 26:85–90, 1967.)

be produced—two complete normal individuals from a single cell. Similarly, one of the cells in a two-cell mouse embryo is destroyed by pricking it with a needle, and the resulting "half-embryo" is placed in the uterus of a foster mother to develop. In many cases a perfectly normal mouse will emerge.

Conversely, two eight-cell mouse embryos can be combined to form a single embryo. This morula, which then develops into a mouse of normal size and structure (Figure 21-85). Such creatures, formed from aggregates of genetically different cells, are called *chimeras*. Chimeras can also be made by injecting cells from an early embryo of one genotype into a blastocyst of another genotype. The injected cells become incorporated into the inner cell mass of the host blastocyst and a chimeric animal develops. A single injected cell taken from an eight-cell embryo or from the inner cell mass of another early blastocyst can give rise to a combination of cell types in the chimera. Wherever the injected cell may go to find itself, it responds correctly to cues from its neighbors and follows the appropriate developmental pathway.

These findings have two implications. First, during the early stages, the developmental system is self-adjusting, so that a normal structure emerges even if the starting conditions are perturbed. Embryos or parts of embryos that have a property are said to be *regulative*. Second, the individual cells of the inner cell mass are initially *totipotent*; they can give rise to any part of the adult body, including germ cells.

Totipotent Embryonic Stem Cells Can Be Obtained from a Mammalian Embryo

A normal early mouse embryo is grafted into the kidney or testis of an adult, its development is disturbed beyond any possibility of proper regulation, but not destroyed. The result is a bizarre tumorous growth known as a *teratoma*, consisting of an organized mass of cells containing many varieties of differentiated tissues—skin, bone, glandular epithelium, and so on—mixed with undifferentiated cells that continue to divide and generate yet more of these differentiated types.

Investigation of the stem cells in teratomas and related types of tumors led to the discovery that their behavior reflects a remarkable property of the cells of the normal inner cell mass: given a suitable environment, they can be induced to proliferate indefinitely while retaining their totipotent character. Cultured cells with this property are called *embryonic stem cells*, or *ES cells*. They can be grown by placing a normal inner cell mass in culture and dispersing the cells as they proliferate. Separating the cells from their normal neighbors and placing them in the appropriate culture medium evidently arrests the normal program of change of cell character with time and so enables the cells to carry on dividing indefinitely without differentiating. Many tissues of the adult body contain stem cells that can divide indefinitely without terminally differentiating, as we shall see in the next chapter; but these *adult stem cells*, when induced to differentiate, normally give rise only to a narrowly restricted range of differentiated cell types.

The state in which the ES cells are arrested seems to be equivalent to that of the normal inner-cell-mass cells. This can be shown by taking ES cells from the cultured inner cell mass and injecting them into a normal blastocyst (Figure 21-86). The injected cells become incorporated in the inner cell mass of the blastocyst and can contribute to the formation of an apparently normal chimeric mouse. Descendants of the injected stem cells can be found in practically any of the tissues of this mouse, where they differentiate in a well-behaved manner appropriate to their position and can even form viable germ cells. The extraordinarily adaptable behavior of ES cells shows that cues from a cell's neighbors not only guide the choice between different pathways of differentiation, but can also stop or start the developmental clock—the processes that drive a cell to progress from an embryonic to an adult state.

On a practical level, ES cells have a two-fold importance. First, from a medical point of view, they offer the prospect of a versatile source of cells for repair

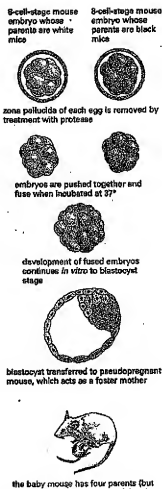


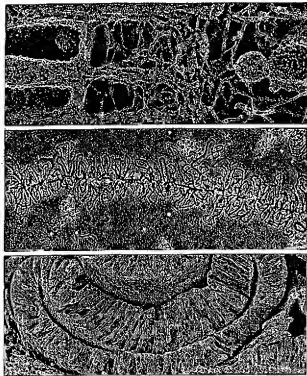
Figure 21-85 A procedure for creating a chimeric mouse. Two morulae of different genotypes are combined.

Developmental Biology

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To Daniel and Sarah

THE COVER

Pluteus larva (front cover) and 32-cell stage (back cover) of the sea urchin *Lytechinus pictus*. Courtesy of George Watchmaker, Lawrence Livermore National Laboratory, Livermore, California.

DEVELOPMENTAL BIOLOGY

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Introduction

According to Aristotle, the first embryologist known to history, science begins with wonder. "It is owing to wonder that people began to philosophize, and wonder remains the beginning of knowledge." The development of animals has been a source of wonder throughout human history, and it has constantly stimulated individuals to seek the causes for such remarkable, yet commonplace, phenomena. The simple procedure of cracking open a chick egg on each day of its 3-week development period provides a remarkable experience—a thin band of cells is seen to give rise to an entire bird. Aristotle performed this experiment and noted the formation of the major organs. Most any person can wonder at this phenomenon, but it is the scientist who seeks to discover how development actually occurs. Rather than dissipating wonder, our new understanding increases it.

Multicellular organisms on earth do not spring forth fully formed. Rather, they arise by a relatively slow process of progressive change that we call DEVELOPMENT. In nearly all cases, the development of a multicellular organism begins with a single cell—the fertilized egg, or ZYGOTE—which divides mitotically to produce all the cells of the body. The study of animal development has traditionally been called EMBRYOLOGY, referring to the fact that between the stage of the fertilized egg and birth, the developing organism is known as an EMBRYO. However, development does not stop at birth—or even at adulthood. Most organisms never cease developing. Each day, we replace over a gram of skin cells (the older cells being sloughed off as we walk), and our bone marrow sustains the development of millions of new erythrocytes every minute of our lives. Therefore, in recent years, it has become customary to speak of DEVELOPMENTAL BIOLOGY as the discipline that involves studies of embryonic and other developmental processes.

Developmental biology is one of the most exciting and fast-growing fields of biology. Part of its excitement comes from its subject matter, for we are just beginning to understand the molecular mechanisms of animal development. Another part of the excitement comes from the unifying role that developmental biology is beginning to assume in the biological sciences. Developmental biology is creating a framework that integrates molecular biology, physiology, cell biology, anatomy, cancer research, immunology, and even evolutionary and ecological studies. The study of development has become essential for understanding any other area of biology.

Principal features of development

Development accomplishes two major functions. It generates the cellular diversity and order within each generation, and it assures the

Introduction

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The first function of life from one generation to the next. The first function of continuity of life from one generation to the next. The first function of continuity of life from one generation to the next. The first function of continuity of life from one generation to the next.

The major features of animal development are illustrated in Figure 1. The life of a new individual is initiated by the fusion of genetic material from the two gametes—the sperm and the egg. This fusion, called *fertilization*, stimulates the egg to begin development. The subsequent sequence of stages is collectively called *embryogenesis*. Throughout the animal kingdom an incredible variety of embryonic types exists, but most patterns of embryogenesis are variations on four themes.

1. Immediately following fertilization, cleavage is a series of extremely rapid mitotic divisions wherein the enormous volume of cytoplasm is divided into numerous smaller cells. These cells are called blastomeres, and by the end of cleavage they generally form a sphere known as a blastula.

2. Under the rule of rabiotic division has slowed down, the blastomeres undergo epiblastic movements wherein they change their positions relative to one another. This series of extensive cell rearrangements is called *GASTRULATION*. As a result of gastrulation, the typical embryo contains three cell regions called *GERM LAYERS*. The outer layer—the *ECOTODERM*—produces the cells of the epidermis and nervous system; the inner layer—the *MESENCHYMA*—produces the lining of the digestive tube and its associated organs (gastrovascular, liver, and so on); and the middle layer—the *GASTRODERM*—gives rise to secretory organs (glands) and the *MESENCHYMA* (connective tissue (bone, muscle, cartilage), and the *ECOTODERM* (epithelia).

3. Once the three germ layers are established, the cells interact with each other and the surrounding environment to produce the bodily organs. This process is called *ontogenogenesis*. (In embryology, *ontogenesis* is introduced when certain mesodermal cells interact with overlying ectodermal cells to cause the latter to form neural tubes. This tube will become the brain and spinal cord.) Many organs contain cells from more than one germ layer, and they are not trusted for the outside of an organ to be derived from one layer and the inside from another. All during *ontogenogenesis* certain cells undergo large migrations from their place of origin to their final location. These migrating cells become the precursors

4. As seen in Figure 1, a portion of egg cytoplasm gives rise to the oil droplet, lymph cells, pigment cells, and gametes.

in *Origin of Species* (1859), "reveals community of descent." In fact, Darwin believed that embryology was the strongest foundation for his evolutionary theory. Von Baer, of course, was not an evolutionist when he wrote his four laws in 1828. In fact, he never became one. Von Baer believed that he had found the divine plan upon which all the organisms in a group such as the vertebrates developed. Because earlier evolutionary theories had envisioned a single, nonbranching series of transformations, von Baer's observations were often used against evolution. Darwin, however, recognized that von Baer's work supported an evolutionary hypothesis in which a common ancestor could radiate into several different types of organisms by heritable modifications of embryonic development. The reason human embryos, fish embryos, and chick embryos all had visceral clefts was because they had a common ancestor whose embryo had such visceral clefts. Moreover, the reason visceral clefts gave rise to different structures in different groups of organisms (gills in fishes, eustachian tubes in mammals) was that the ancestral plan had been modified through the action of natural selection.

Von Baer also recognized that there was a common pattern to vertebrate development. The three germ layers gave rise to different organs, and their derivation was constant whether the organism was a fish, a frog, or a chick. ECTODERM formed skin and nerves; ENDODERM formed respiratory and digestive tubes; and MESODERM formed connective tissue, blood cells, heart, the urogenital system, and parts of most of the internal organs. In this chapter we shall follow the early development of ectoderm, focusing on formation of the nervous system. In the next chapter we shall follow the early development of endodermal and mesodermal organs.

Neurulation

In vertebrates, gastrulation creates an embryo having an internal endodermal layer, an intermediate mesodermal layer, and an external ectoderm. In addition, a cord of mesodermal cells, the notochord, lies directly beneath the most dorsal portion of the ectoderm. The interaction between the notochord and its superadjacent ectoderm is one of the most important interactions of all development, for the notochord directs the ectoderm to form the hollow NEURAL TUBE, which will differentiate into the brain and spinal cord. Thus, we begin a new phase of development—ORGANOGENESIS, the creation of tissues and organs. The action by which the notochord instructs the ectoderm to become neural tube is called PRIMARY EMBRYONIC INDUCTION, and the cellular response by which the flat layer of ectodermal cells is transformed into a hollow tube is called NEURULATION. The events of neurulation are diagrammed in Figure 2. Here, the original ectoderm is divided into three sets of cells: (1) the internally positioned neural tube, (2) the epidermis of the skin, and (3) the neural crest cells, which migrate from the region that had connected the neural tube and epidermal tissues.

Early vertebrate development:
mesoderm and endoderm

Not physiognomy alone or brain alone is worthy for the Muse,
I say the form complete is essential for

—WALT WHITMAN (1867)

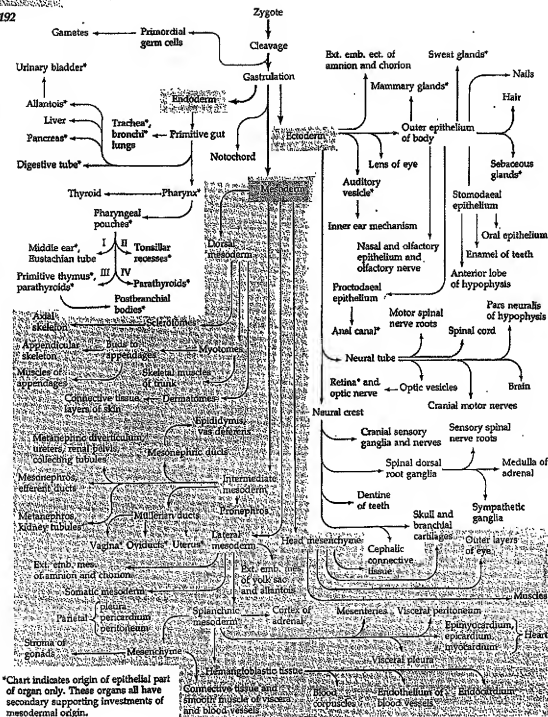
The greatest progressive minds of entomology have not searched for hypotheses; they have looked at embryos.

—Л. М. ОРЕНБУМЕР (1955)

In the last chapter we followed the various tissues formed by developing the endoderm. In this chapter, we shall follow the early development of the mesodermal and endodermal germ layers. Endoderm will be seen to form the lining of the digestive and respiratory tracts with their associated organs; mesoderm will be seen to generate all the organs between the ectodermal wall and the endodermal tissues.

The positions of the remaining sensory ganglia can be divided into three categories. The first category is the craniosacral ganglia. These ganglia are located in the cranial and sacral regions of the nervous system. The second category is the thoracic ganglia. These ganglia are located in the thoracic region of the nervous system. The third category is the lumbar ganglia. These ganglia are located in the lumbar region of the nervous system.

One of the major tasks of gastrulation is to position the endoderm deep within the embryo and to sandwich the mesodermal cells between the ectoderm and endoderm. As shown in Figure 2, the formation of mesodermal and endodermal organs is not subsequent to normal tube formation, but occurs precursively. These mesodermal cells of the foregut that are not involved in notochord formation have migrated laterally to form thick bands running lengthwise along each side of the notochord and neural tube. These bands are called paraxial mesoderm. At the primitive streak regressions and the neural folds begin to grow out of the center of the embryo, the paraxial mesoderm separates into triangular blocks of cells called somites. The first somites appear in the anterior portion of the embryo, and new somites are formed posteriorly



Development of blood cells

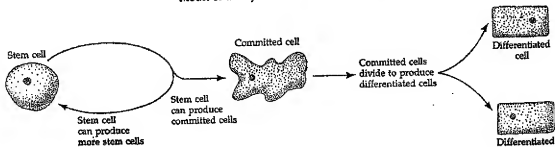
Pluripotential stem cells
and hematopoietic microenvironments

Most vertebrate tissues are composed of differentiated cells that no longer divide. Myoblasts, for instance, are a rapidly dividing cell population until they develop into myotubes. Some tissues, such as epidermis, intestinal epithelium, and blood cells, however, retain an "embryonic" cell population within themselves, such that their cellular composition is always changing, even in adult animals. This is most evident in the case of the mammalian red blood cell. This cell lacks a nucleus and has a life span of only 120 days in circulation. A normal person will lose and replace 3×10^{11} red blood cells every day (Hay, 1966). The continuous formation of new red blood cells (as well as all the other types of blood cells) is accomplished in the bone marrow by the HEMATOPOIETIC ("blood-forming") STEM CELLS.

Stem cells are an intriguing and little-understood phenomenon. Yet our lives depend upon them. A stem cell is a cell that is capable of extensive proliferation and that can generate more stem cells ("self-renewal") as well as more differentiated progeny (Siminovitch et al., 1963). Thus, a single stem cell can generate a clone containing millions of differentiated cells as well as a few stem cells. The notion of a stem cell is depicted in Figure 25.

In mammals and birds, there appears to be a common FLURIPOTENTIAL HEMATOPOIETIC STEM CELL, which can give rise to red blood cells (erythrocytes), white blood cells (granulocytes), macrophages, platelets, and immunocompetent cells (lymphocytes). The existence of such stem cells was shown by Till and McCulloch (1961), who injected bone mar-

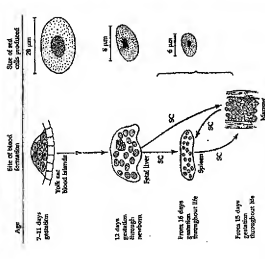
FIGURE 25
Model of the dynamics of stem cell proliferation and differentiation.



Sites of hematopoiesis

In most mammals, the major source of blood cells in the adult is the bone marrow. In some species, like the mouse, the spleen also participates. However, numerous experiments have shown that the hematopoietic stem cells do not originate in the marrow or spleen, but rather, they migrate there from other regions of the embryo. In fact, the mammalian hematopoietic stem cell probably comes from outside the mammalian blastocyst, and is derived from the yolk sac of the embryo proper, that is, from the yolk sac. When Moore and his co-workers removed the yolk sacs from presomite mouse embryos, no hematopoietic development took place in the embryo. Moreover, in

FIGURE 28
The successive sites of blood formation in the embryonic mouse. Arrows indicate potential migratory paths of pluripotential hematopoietic stem cells (SC). The comparative size of the red blood cells produced in each site is shown at the right. (After Russell, 1979.)



5. EARLY VERTEBRATE DEVELOPMENT: MESODERM AND ENDODERM

numbers of the isolated yolk sac endodermis and CEMs production of stem cells. Morrison and Melnick (1977) demonstrated that the peak of CEM activity in the yolk sac was at 10 days. In contrast, the embryonic endodermis was found to have CEM activity only after 11 days. Thus, it was concluded that the stem cells migrate from the yolk sac to the endodermis at 10 days and then to the spleen and bone marrow. Embryonic yolk sac cells have also been seen to carry out lymphopoietic functions (Morrison and Melnick, 1977; Morrison and Melnick, 1978; Morrison and Gelfond, 1978; Dahl et al., 1980). Thus, the original nanoblastic pluripotential stem cell is thought to arise within the yolk sac and migrate first to the liver and then to the adult hematopoietic organs (Figure 2).

A similar situation was believed to occur in adult embryos. The first is in mammalian embryos located in the yolk sac. These cells, which are located in the yolk sac, are the first to be affected by the virus. The second is in the blood islands, which form within the embryonic proper. This was discovered in a series of elegant experiments by Olsnes and Luzzo, who perfused the hindbrain of chicken embryos with the virus. They found that the cells within the yolk sac and the blood islands were the first to be infected and that the cells within the embryonic proper were the last to be infected. This suggests that the virus enters the embryo through the yolk sac and the blood islands and then spreads to the rest of the embryo.

ENDODERM

Pharynx

The function of embryonic endostem is to construct the linings of two tubes within the body. The first tube, extending throughout the length of the body, is the digestive tube. Buds from this tube form the liver, gall bladder, and pancreas. The second tube is the respiratory tube. This tube eventually bifurcates into two lungs. The digestive and respiratory tubes share a common chamber in the anterior region of the embryo, and this region is called the **pharynx**. Epithelial components of the pharynx give rise to the tonsils, thyroid, thymus, and parathyroid glands.

EMBRYONIC DEVELOPMENT

Embryonic

...the major source of blood cells in the adult is the ... (in some species, like the mouse, the spleen also produces ... cells do not originate in the marrow or spleen, but ... from other regions of the embryo. In fact, ... embryonic stem cell probably serves from ... the ... this ... from precursors mouse embryos, no ... body plan in the embryo. Moreover, in

... formation in the embryonic mouse. Arrows indicate ... of pluripotential hematopoietic stem cells (HSC), ... cells produced in cells are shown at



6. EARLY VEGETATIVE DEVELOPMENT: MESODERM AND ENDODERM

cultures of the isolated yolk sac, erythropoietin and CFU-E production ... CFU-E activity in this yolk sac was at 10 days. In contrast, the embryonic liver begins to show the presence of stem cells only after 11 days. Thus, it was postulated that the stem cells migrate from the yolk sac to the liver. ... yolk sac cells have also been seen to carry out lymphopoietic functions before any stem cell activity is present within the embryo proper (Baltimore and Chishti, 1979; ... cell is the first to appear within the yolk sac and migrate first to the liver and then to the adult hematopoietic organs (Figure 29).

A similar situation was believed to occur in avian embryos, but birds were thought to have two separate sources of hematopoietic stem cells. The first is, as in mammalian embryos, located in the yolk sac. These cells, however, appear to be a transient population. The major source of stem cells in the avian embryo is the blood islands that develop within the embryo proper. This was discovered in a series of elegant experiments by Davidson-Lere, who grafted the blastoderm of chicken embryos of the Japanese quail (Figure 29). Chick cells can be distinguished from quail cells by the presence of a nucleolar pattern that is much more clearly providing a permanent marker for distinguishing the two cell types. Using these "yolk sac chickens," Davidson-Lere and Melnick (1975) have shown that the stem cells of the avian embryo migrate only to the adult animal but that the true stem cells are formed within nodules of mesoderm that line the mesentery and the major blood vessels. In mammals, the yolk sac is still thought to be the source of stem cells (Davidson-Lere and Melnick, 1975; Davidson-Lere and Amlach, 1983) have suggested that these yolk sac blood precursors may be derived from stem cells lining the abdominal cavity.

ENDODERM

Pharynx

The function of embryonic endoderm is to construct the linings of two tubes within the body. The first tube, extending throughout the length of the body, is the digestive tube. Budd from this tube form the lungs, the trachea, and the thyroid gland. The second tube is the respiratory tube. This tube eventually branches into two lungs. The digestive and respiratory tubes share a common chamber in the anterior region of the embryo, and this region is called the pharynx. The pharynx is the site where the digestive and respiratory tubes give rise to the larynx, thyroid, lungs, and parathyroid glands.

Appendix C

99. A cell culture comprising isolated expanded human multipotent, non-embryonic, non-germ, cells that can differentiate into at least one cell type of each of the endodermal, ectodermal, and mesodermal embryonic lineages and express telomerase, said cells having undergone at least 10-40 cell doublings in culture.

100. A cell culture comprising isolated expanded human multipotent, non-embryonic, non-germ, cells that can differentiate into at least one cell type of each of the endodermal, ectodermal, and mesodermal embryonic lineages, express telomerase, and have been obtained by culture of non-embryonic, non-germ tissue, the telomerase-expressing cells having undergone at least 10-40 cell doublings in culture.

101. The cell culture of claim 99 or 100 wherein the telomerase-expressing cells have undergone 10 cell doublings.

102. The cell culture of claim 99 or 100 wherein the telomerase-expressing cells have undergone 20 cell doublings.

103. The cell culture of claim 99 or 100 wherein the telomerase-expressing cells have undergone 30 cell doublings.

104. The cell culture of claim 99 or 100 wherein the telomerase-expressing cells have undergone 40 cell doublings.

105. The cell culture of claim 99 or 100, wherein the cells are genetically modified.

106. The cell culture of claim 105, wherein the modification comprises introducing a selectable or screenable marker gene into the cells.

107. A pharmaceutical composition comprising the telomerase-expressing cells of claim 99 or 100, in a pharmaceutically acceptable carrier, the telomerase-expressing cells obtained from the cell culture of claim 99 or 100.

108. A method for making a pharmaceutical composition comprising admixing the telomerase-expressing cells of claim 99 or 100 with a pharmaceutically acceptable carrier, the telomerase-expressing cells obtained from the cell culture of claim 99 or 100.

109. A method for making the cell culture of claim 99 or 100, said method comprising introducing the telomerase-expressing cells into cell culture medium and expanding said cells such that said cells undergo at least 10-40 cell doublings in culture.

110. The cell culture of claim 99 or 100 wherein the telomerase-expressing cells have undergone greater than 40 cell doublings.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-283. (Canceled)

284. (New) A method for identifying an agent that is a differentiation factor, the method comprising:

(a) contacting (1) a desired agent with (2) isolated expanded human multipotent, non-embryonic, non-germ, cells that can differentiate into at least one cell type of each of the endodermal, ectodermal and mesodermal embryonic lineages and express telomerase; and

(b) determining whether the agent of step (a) affects differentiation of the telomerase-expressing cells into a desired differentiated progeny, where affecting differentiation indicates an agent that is a differentiation factor.

285. (New) The method of claim 284 wherein the telomerase-expressing cells express oct3/4.

286. (New) The method of claim 284, wherein the telomerase-expressing cells have undergone at least 10-40 cell doublings.

287. (New) The method of claim 284, wherein differentiation is into an endodermal cell type.

288. (New) The method of claim 284, wherein differentiation is into a mesodermal cell type.

289. (New) The method of claim 284, wherein differentiation is into an ectodermal cell type.

290. (New) The method of claim 287, wherein the endodermal cell type is hepatic or pancreatic.

291. (New) The method of claim 288, wherein the mesodermal cell type is osteoblast, chondrocyte, bone, adipocyte, fibroblast, marrow stroma, skeletal muscle, smooth muscle, cardiac muscle, endothelial, epithelial, or hematopoietic.

292. (New) The method of claim 289, where the ectodermal cell type is astrocyte, glial, neuronal, or oligodendrocyte.
293. (New) The method of claim 284 wherein the telomerase-expressing cells are genetically modified.
294. (New) The method of claim 293, wherein the telomerase-expressing cells are modified to contain a selectable or screenable marker gene.
295. (New) The method of claim 284, wherein the telomerase-expressing cells have undergone greater than 40 cell doublings.
296. (New) The method of claim 284, wherein the effect on differentiation is assayed by changes in protein expression, RNA expression, or morphology.
297. (New) A method for identifying an agent that is a differentiation factor, the method comprising determining whether an agent affects differentiation, into a desired differentiated progeny, of isolated expanded human multipotent, non-embryonic, non-germ, cells that can differentiate into at least one cell type of each of the endodermal, ectodermal, and mesodermal embryonic lineages and express telomerase.
298. (New) The method of claim 297 wherein the telomerase-expressing cells express oct3/4.
299. (New) The method of claim 297, wherein the telomerase-expressing cells have undergone at least 10-40 cell doublings.
300. (New) The method of claim 297, wherein differentiation is into an endodermal cell type.
301. (New) The method of claim 297, wherein differentiation is into an ectodermal cell type.
302. (New) The method of claim 297, wherein differentiation is into a mesodermal cell type.

Appendix D

cells for therapies directed to the majority of the population (Wadman, M.,
Nature (1999) 398: 551).

Using cells from the developed individual, rather than an embryo, as a
source of autologous or allogeneic stem cells would overcome the problem of
tissue incompatibility associated with the use of transplanted embryonic stem
cells, as well as solve the ethical dilemma associated with embryonic stem cell
research. The greatest disadvantage associated with the use of autologous stem
cells for tissue transplant thus far lies in their limited differentiation potential. A
number of stem cells have been isolated from fully-developed organisms,
particularly humans, but these cells, although reported to be multipotent, have
demonstrated limited potential to differentiate to multiple cell types.

Thus, even though stem cells with multiple differentiation potential have
been isolated previously by others and by the present inventors, a progenitor cell
with the potential to differentiate into a wide variety of cell types of different
lineages, including fibroblasts, osteoblasts, chondrocytes, adipocytes, skeletal
muscle, endothelium, stroma, smooth muscle, cardiac muscle and hemopoietic
cells, has not been described. If cell and tissue transplant and gene therapy are to
provide the therapeutic advances expected, a stem cell or progenitor cell with the
greatest or most extensive differentiation potential is needed. What is needed is
the adult equivalent of an embryonic stem cell.

Summary of the Invention

The present invention provides an isolated multipotent mammalian stem
cell that is surface antigen negative for CD44, CD45, and HLA Class I and II.
The cell may also be surface antigen negative for CD34, Muc18, Stro-1, HLA-
class-I and may be positive for oct3/4 mRNA, and may be positive for hTERT
mRNA. In particular, the cell may be surface antigen negative for CD31, CD34,
CD36, CD38, CD45, CD50, CD62E and CD62P, HLA-DR, Muc18, STRO-1,
cKit, Tie/Tek, CD44, HLA-class I and 2-microglobulin and is positive for
CD10, CD13, CD49b, CD49e, CDw90, Flk1, EGF-R, TGF-R1 and TGF-R2,
BMP-R1A, PDGF-R1a and PDGF-R1b. The present invention provides an
isolated multipotent non-embryonic, non-germ cell line cell that expresses

transcription factors oct3/4, REX-1 and ROX-1. It also provides an isolated multipotent cell derived from a post-natal mammal that responds to growth factor LIF and has receptors for LIF.

The cells of the present invention described above may have the capacity to be induced to differentiate to form at least one differentiated cell type of mesodermal, ectodermal and endodermal origin. For example, the cells may have the capacity to be induced to differentiate to form cells of at least osteoblast, chondrocyte, adipocyte, fibroblast, marrow stroma, skeletal muscle, smooth muscle, cardiac muscle, endothelial, epithelial, hematopoietic, glial, neuronal or oligodendrocyte cell type. The cell may be a human cell or a mouse cell. The cell may be from a fetus, newborn, child, or adult. The cell may be derived from an organ, such as from marrow, liver or brain.

The present invention further provides differentiated cells obtained from the multipotent adult stem cell described above, wherein the progeny cell may be a bone, cartilage, adipocyte, fibroblast, marrow stroma, skeletal muscle, smooth muscle, cardiac muscle, endothelial, epithelial, endocrine, exocrine, hematopoietic, glial, neuronal or oligodendrocyte cell. The differentiated progeny cell may be a skin epithelial cell, liver epithelial cell, pancreas epithelial cell, pancreas endocrine cell or islet cell, pancreas exocrine cell, gut epithelium cell, kidney epithelium cell, or an epidermal associated structure (such as a hair follicle). The differentiated progeny cell may form soft tissues surrounding teeth or may form teeth.

The present invention provides an isolated transgenic multipotent mammalian stem cell as described above, wherein genome of the cell has been altered by insertion of preselected isolated DNA, by substitution of a segment of the cellular genome with preselected isolated DNA, or by deletion of or inactivation of at least a portion of the cellular genome. This alteration may be by viral transduction, such as by insertion of DNA by viral vector integration, or by using a DNA virus, RNA virus or retroviral vector. Alternatively, a portion of the cellular genome of the isolated transgenic cell may be inactivated using an antisense nucleic acid molecule whose sequence is complementary to the sequence of the portion of the cellular genome to be inactivated. Further, a